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Morphological synergism in root hair length, density, initiation and geometry for phosphorus acquisition in *Arabidopsis thaliana*: A modeling approach

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Abstract

Low phosphorus availability regulates root hair growth in *Arabidopsis* by (1) increasing root hair length, (2) increasing root hair density, (3) decreasing the distance between the root tip and the point at which root hairs begin to emerge, and (4) increasing the number of epidermal cell files that bear hairs (trichoblasts). The coordinated regulation of these traits by phosphorus availability prompted us to speculate that they are synergistic, that is, that they have greater adaptive value in combination than they do in isolation. In this study, we explored this concept using a geometric model to evaluate the effect of varying root hair length (short, medium, and long), density (0, 24, 48, 72, 96, and 120 root hairs per mm of root length), tip to first root hair distance (0.5, 1, 2, and 4 mm), and number of trichoblast files (8 vs. 12) on phosphorus acquisition efficiency (PAE) in *Arabidopsis*. *SimRoot*, a dynamic three-dimensional geometric model of root growth and architecture, was used to simulate the growth of *Arabidopsis* roots with contrasting root hair parameters at three values of phosphorus diffusion coefficient (D_e=1×10^{-7}, 1×10^{-8}, and 1×10^{-9} cm^2 s^{-1}) over time (20, 40, and 60 h). *Depzone*, a program that dynamically models nutrient diffusion to roots, was employed to estimate PAE and competition among root hairs. As D_e decreased from 1×10^{-7} to 1×10^{-9} cm^2 s^{-1}, roots with longer root hairs and higher root hair densities had greater PAE than those with shorter and less dense root hairs. At D_e=1×10^{-9} cm^2 s^{-1}, the PAE of root hairs at any given density was in the order of long hairs > medium length hairs > short hairs, and the maximum PAE occurred at density = 96 hairs mm^{-1} for both long and medium length hairs. This was due to greater competition among root hairs when they were short and dense. Competition over time decreased differences in PAE due to density, but the effect of length was maintained, as there was less competition among long hairs than short hairs. At high D_e(1×10^{-7} cm^2 s^{-1}), competition among root hairs was greatest among long hairs and lowest among short hairs, and competition increased with increasing root hair densities. This led to a decrease in PAE as root hair length and density increased. PAE was also affected by the tip to first root hair distance. At low D_e values, decreasing tip to first root hair distance increased PAE of long hairs more than that of short hairs, whereas at high D_e values, decreasing tip to first root hair distance increased PAE of root hairs at low density but decreased PAE of long hairs at very high density. Our models confirmed the benefits of increasing root hair density by increasing the number of trichoblast files rather than decreasing the trichoblast length. The combined effects of all four root hair traits on phosphorus acquisition was 371% greater than their additive effects, demonstrating substantial morphological synergy. In conclusion, our data support the hypothesis that the responses of root hairs to low phosphorus availability are synergistic, which may account for their coordinated regulation.

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Introduction

Plant root hairs are subcellular extensions from the root epidermis that facilitate the acquisition of immobile nutrients such as phosphorus, by increasing the absorptive surface area of the root and allowing the root to explore a greater soil volume. The importance of root hairs in the acquisition of phosphorus has been shown by the observation of the depletion zones of ^{32}P around root hairs (Bhat and Nye, 1974; Lewis and Quirk, 1967). Research shows that among barley cultivars, greater absorption of soil phosphorus is associated with longer and denser root hairs (Gahoonia and Nielsen, 1997). In *Arabidopsis*, comparisons of wild type and hairless mutants showed the importance of root hairs for phosphorus acquisition (Bates and Lynch, 2000a) and phosphorus acquisition efficiency (Bates and Lynch, 2000b).

Many plant species exhibit increased extension of root hairs in response to phosphorus deficiency (Bates and Lynch, 1996; Fohse and Jungk, 1983). In *Arabidopsis*, root hairs grow longer and more dense under low phosphorus, and phosphorus acquisition per unit root length is increased with longer root hairs (Bates and Lynch, 1996, 2000b; Ma et al., 2001). The increased root hair density caused by low phosphorus availability is partly due to anatomical changes leading to an increased number of trichoblast files (Ma et al., 2001). In addition, the distance from the root tip to the first root hair decreases (Zhang et al., unpublished). In *Arabidopsis*, low phosphorus availability regulates these four traits in a coordinated manner. Various studies have reported that the effects of phosphorus availability on root hair length and density are correlated; treatments that lead to longer hairs also lead to increased root hair density (Bates and Lynch, 1996; Gahoonia et al., 1997; Ma et al., 2001; Yan et al., unpublished). The coordinated expression of these traits may indicate common regulatory control mechanisms, and may also indicate functional synergism. In other words, the adaptive value of these traits for phosphorus acquisition may be greater in the aggregate than the additive effect of each trait in isolation.

In contrast to nutrients that move via mass flow, such as nitrate, phosphorus mobility in soil is governed by diffusion, which makes its acquisition very dependent on the temporal and spatial exploration of the soil by the root system (Barber, 1995). Inter-root competition, an important component of the nutrient acquisition efficiency of root systems (Nielsen et al., 1994), occurs when the same volume of soil is explored by adjacent roots of the same plant. Root systems with less spatial overlap in the depletion volume of adjacent roots will have less inter-root competition, and therefore tend to be more efficient, i.e. will expend less root resources in phosphorus acquisition. Under low phosphorus availability, the increased length and density of root hairs in *Arabidopsis* will increase the total surface area of the root and extend soil exploration by the root system, while at the same time, increasing competition among root hairs due to overlapping zones of soil exploration. To what extent the ‘cost’ from possible competition among root hairs is balanced by the ‘benefit’ from increased soil exploration remains an open question.

Although the responses of *Arabidopsis* root hairs to phosphorus availability have been characterized (Bates and Lynch, 1996; Ma et al., 2001), little is known about how root hair parameters (length, density, tip to first root hair distance and geometry) coordinate with each other for optimal phosphorus acquisition. One obstacle to this effort is the difficulty of measuring nutrient gradients with the precision needed to determine the extent and overlap of depletion volumes in a three dimensional context. In this regard, geometric modeling has been a useful tool in simulating and understanding the functional properties of root systems, such as water acquisition (Clausnitzer and Hopmans, 1994; Doussan et al., 1998), water and nutrient acquisition (Somma et al., 1998), exploitation efficiency (Fitter et al., 1991), and inter-root competition and phosphorus acquisition efficiency (Ge et al., 2000).

SimRoot is a dynamic geometric model of root systems based on empirical growth parameters (Lynch et al., 1997). In this study, we used *SimRoot* to evaluate the effect of varying root hair parameters on phosphorus acquisition efficiency in *Arabidopsis*. Our premise is that phosphorus acquisition efficiency is enhanced by root hair traits that maximize soil exploration while minimizing spatial competition among root hairs. The specific hypotheses tested were: (1) that increased root hair length and increased root hair density are synergistic in increasing phosphorus acquisition, (2) that the value of the diffusion coefficient for phosphorus (D_e) is an important determinant of soil exploration and competition among root hairs and therefore phosphorus acquisition efficiency; (3) that shortened tip to first root hair distance increases soil exploration and phosphorus acquisition; and (4) that increasing root hair density through developing more

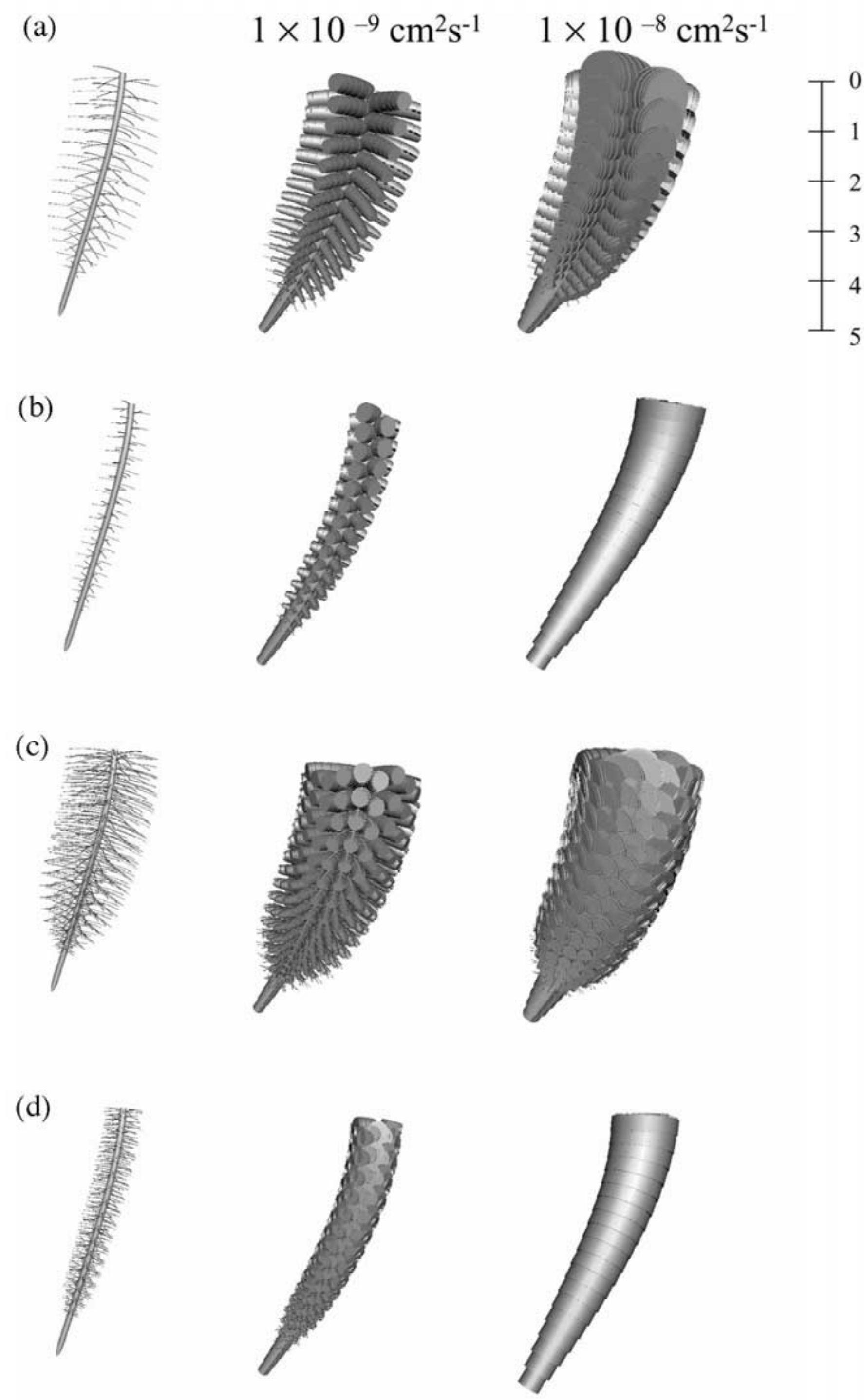

Figure 1. Simulated *Arabidopsis* taproots with root hairs differing in length and density. Simulation time was 20 h. Models were parameterized with real measurements of wild type *Arabidopsis* roots. Reference axis: 0 – 5 cm. The leftmost column shows root models with long or short hairs at two levels of density. (a) long hairs, density = 24; (b) short hairs, density =24; (c) long hairs, density = 96; (d) short hairs, density = 96. The same root model with depletion volumes present at different D_e values is shown on each row from left to right, with the one on the left at D_e=1×10^{-9} cm^2 s^{-1} and the one on the right at D_e=1×10^{-8} cm^2 s^{-1}. As D_e increases to 1×10^{-7} cm^2 s^{-1} the depletion volumes become cones for all models.

Table 1. Root hair growth parameters used in the simulations

1. Root hair length (mm)[a]		long:	0.87	medium:	0.47		short:	0.29
2. Root hair density[b]								
(no. of root hairs/mm)			0	24	48	72	96	120
3. Tip to first root								
hair distance (mm)[c]			0.5	1	2	4		
4. Root hair growth								
rate (mm/h)[a]	long:		0.18, 0.20, 0.16, 0.14, 0.10, 0.05, 0.03, 0.01, 0					
	medium:		0.15, 0.14, 0.11, 0.06, 0.01, 0					
	short:		0.12, 0.08, 0.06, 0.02, 0.01, 0					

[a]Values based on Bates and Lynch (1996).
[b]Values based on Ma et al. (2001).
[c]Values based on Zhang et al. (unpublished).

trichoblast files is more beneficial than decreasing trichoblast length.

Materials and methods

Description of root hair model

SimRoot was modified to describe the morphology and growth parameters of wild type *Arabidopsis thaliana* roots and root hairs. The basic root hair model simulated was that of *Arabidopsis thaliana* L. (Heynh), ecotype ‘Columbia’ (Figure 1). Root hair growth parameters for the models (Table 1) were taken from studies on root hair responses to phosphorus availability (Bates and Lynch, 1996; Ma et al., 2001). Each simulation model was replicated three times by changing the seed of the random number generator used to reproduce stochastic processes in the growth of each root system, resulting in roots with the same overall morphology but some degree of randomness. This variation simulates phenotypic variation in root growth.

Depletion volume and competition among root hairs for phosphorus

The phosphorus depletion volume of the whole root system was divided into a group of elements, with each element represented by a voxel, or cubic-form volume, within the depletion cylinder (Ge et al., 2000). The size of voxels was negatively correlated with resolution in the calculations of depletion volumes. In our simulations the volume of each voxel was 0.001 mm^3, which had reasonable resolution (variation in less than 5% occurred with an 88% decrease in voxel size). The radius of the depletion zone volume can be expressed as:

$$R_{\mathrm{dz}} = r + \sqrt{D_e t}, \qquad (1)$$

where R_{dz} is the radius of depletion zone around the root as measured from the center of the root; r is the radius of the root segment; D_e is the diffusion coefficient of phosphorus and t is the time period of root growth (Fitter et al., 1991; Ge et al., 2000; Nye and Tinker, 1977; Tinker and Nye, 2000).

Since each root hair has its own depletion cylinder which can overlap with those of other root hairs, the sum of the depletion volumes of all the root hairs overestimates the actual depletion volume of the whole root system. The overlap volume indicates competition for phosphorus among root hairs. A relative index was used to calculate the extent of this competition, as:

$$\text{Competition} = \frac{V_t - V\mathrm{a}}{V\mathrm{a}} 100\%, \qquad (2)$$

where V_t is the total depletion volume including the overlap and V_{a} is the actual depletion volume.

Phosphorus acquisition efficiency

Since the carbon cost involved in constructing root hairs has not been determined precisely, in this study we defined phosphorus acquisition efficiency (PAE) as:

$$\mathrm{PAE} = \frac{V\mathrm{a}}{V\mathrm{r}} 100\%, \qquad (3)$$

where *V*a is the actual depletion volume representing the ‘benefit function’, *V*r is the total root volume representing the ‘cost function’, and it is assumed that all available phosphorus in the depletion is incorporated into the root system.

Variation in the phosphorus diffusion coefficient

We used phosphorus diffusion coefficient values of 1×10^{-7}, 1×10^{-8}, and 1×10^{-9} cm^2 s^{-1}, which spans the range of values found in most field conditions as reported by Schenk and Barber (1979). The higher D_e is close to that found in an Aquic Argiudoll and the lower to a Typic Udipsamment (Schenk and Barber, 1979).

Data analysis

Statistical analyses of the data were conducted using SAS (Statistical Analysis Systems Institute, 1982). Randomized-block ANOVA and Waller-Duncan K-ratio t-test were used to compare data generated from different models over the time course of the simulation. Regression analysis was performed to study the correlation between root hair length and density. Probabilities of 0.05 or less were considered to be statistically significant.

Additional assumptions

To simplify the modeling process, we made the following assumptions: (1) water and other nutrients have no interactions with phosphorus on competition among root hairs; (2) the effects of phosphorus-mobilizing root exudates such as protons or organic acids on phosphorus mobility in the depletion zone are negligible; (3) phosphorus in the overlapped depletion volumes is equally shared by all competing root hairs; (4) all root surfaces are equally active in phosphorus acquisition; and (5) competition between roots of neighboring plants is ignored.

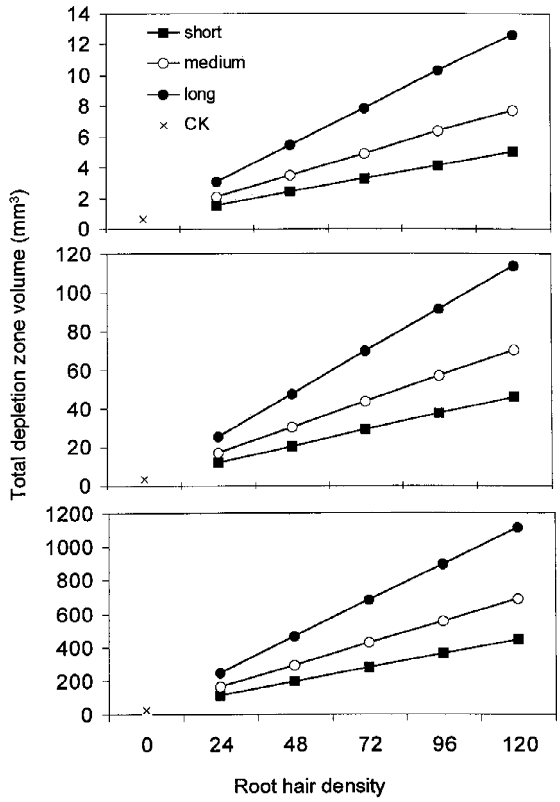

Figure 2. Total depletion volumes of roots with root hairs at various lengths and densities under three diffusion coefficient values (from top down, D_e=1×10^{-9}, 1×10^{-8}, and 1×10^{-7} cm^2 s^{-1}). Control (CK) represents a single root with no root hairs. Data shown as mean ± standard error of the mean (n = 3). Error bars not shown where their size is smaller than that of the symbols. ANOVA indicates significant effects of root hair length (P<0.0001) and density (P<0.0001), and interaction between the two (P<0.0001) under all D_e values. Waller-Duncan K-ratio t-test shows significant differences among different models (K-ratio=100). For D_e=1×10^{-9}, F=22370, critical value of t is 1.81, and minimum significant difference is 0.056. For D_e=1×10^{-8}, F=204324, critical value of t is 1.81, and minimum significant difference is 0.169. For D_e=1×10^{-7}, F=1420136, critical value of t is 1.81, and minimum significant difference is 0.632.

Results

Phosphorus depletion volumes

Roots with root hairs had greater overall phosphorus depletion volume than single roots with no root hairs. Total phosphorus depletion volume increased linearly as root hair density increased for root hairs of all lengths at all three phosphorus diffusion coefficients (Figure 2). As D_e increased from 1×10^{-9} to 1×10^{-7} cm^2 s^{-1}, the rate of expansion in total depletion volume increased with root hair density by a factor of 10 at any given root hair length. At each D_e value, long root hairs expanded the total depletion volume 1.6 times faster than medium length hairs, and 2.6 times faster than short hairs (Figure 2). Actual depletion volume (i.e. taking into account depletion volume overlap) was also smallest around roots with no root hairs (Figure 3). The largest actual depletion zone volumes were observed at the highest D_e value (1×10^{-7} cm^2 s^{-1}), at which there was little effect of increasing root hair length or density (Figure 3). As D_e decreased, increases in root hair length and density had increasingly significant effects on actual depletion volume, and the effect of combining increased density with increased length was greater than the sum or product of increasing each separately (Figure 3).

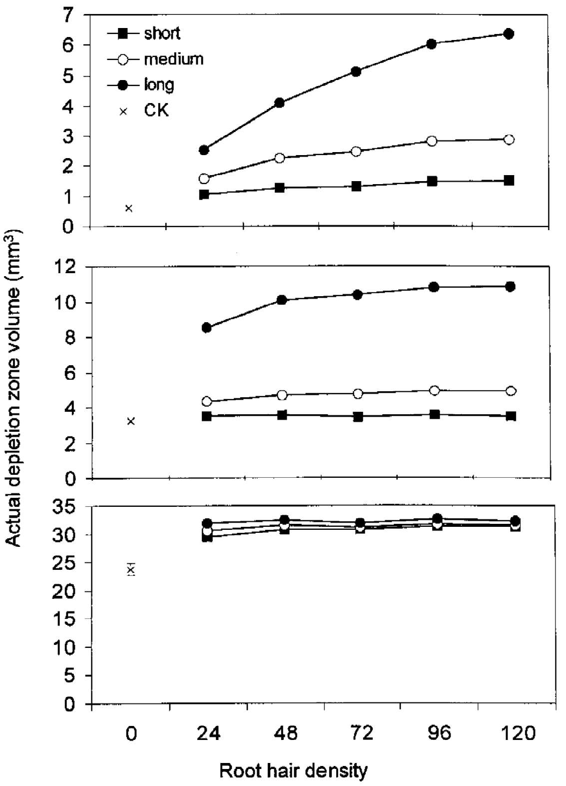

Figure 3. Actual depletion volumes of roots with root hairs at various lengths and densities under three diffusion coefficient values (from top down, D_e=1$\times$10^{-9}, 1$\times$10^{-8}, and 1$\times$10^{-7} cm^2 s^{-1}). Control (CK) represents a single root with no root hairs. Data shown as mean $\pm$ standard error of the mean (n = 3). Error bars not shown where their size is smaller than that of the symbols. ANOVA indicates significant effects of root hair length (P<0.0001) and density (P<0.0001) under all D_e values, and significant interaction between the two (P<0.0001) except under D_e=1$\times$10^{-7} cm^2 s^{-1}. Waller-Duncan K-ratio t-test shows significant differences among different models (K-ratio=100). For D_e=1$\times$10^{-9}, F=29334, critical value of t is 1.81, and minimum significant difference is 0.027. For D_e=1$\times$10^{-8}, F=118769, critical value of t is 1.81, and minimum significant difference is 0.0223. For D_e=1$\times$10^{-7}, F=65.11, critical value of t is 1.82, and minimum significant difference is 0.654.

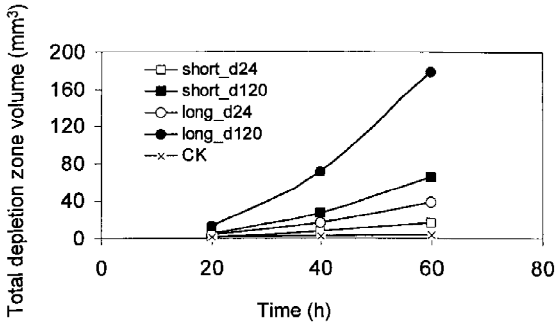

Figure 4. Total depletion volumes through time of roots with long or short root hairs at either high or low densities. D_e=1$\times$10^{-9} cm^2 s^{-1}. Control (CK) represents a single root with no root hairs. Data shown as mean $\pm$ standard error of the mean (n = 3). Error bars not shown where their size is smaller than that of the symbols. ANOVA indicates significant effect of time (P<0.0001) and its interaction with length (P<0.0001) and density (P<0.0001). Waller-Duncan K-ratio t-test shows significant differences among different models at different time points (K-ratio=100): F=19300, critical value of t is 1.81, and minimum significant difference is 0.865.

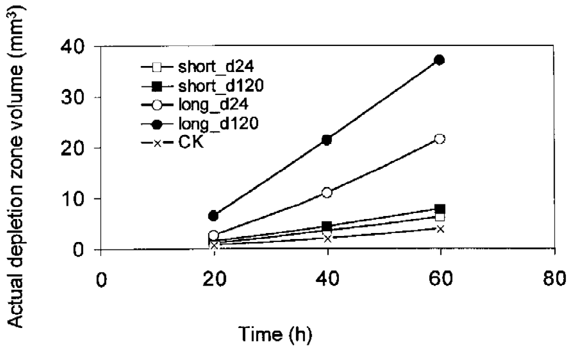

Figure 5. Actual depletion volumes through time of roots with long or short root hairs at either high or low densities. D_e=1$\times$10^{-9} cm^2 s^{-1}. Control (CK) represents a single root with no root hairs. Data shown as mean $\pm$ standard error of the mean (n = 3). Error bars not shown where their size is smaller than that of the symbols. ANOVA indicates significant effect of time (P<0.0001) and its interaction with length (P<0.0001) and density (P<0.0001). Waller-Duncan K-ratio t-test shows significant differences among different models at different time points (K-ratio=100): F=3111, critical value of t is 1.81, and minimum significant difference is 0.471.

At the lowest D_e(1$\times$10^{-9} cm^2 s^{-1}), the growth of roots having higher root hair density increased the total volume of the depletion zone faster than that with low density of root hairs over the course of 60 h (Figure 4). At both the highest and lowest root hair densities, long root hairs achieved greater and faster expansion of the total depletion volume than short hairs as they grew. Actual depletion volume increased linearly at a much faster rate with growth of roots of long root hairs at higher root hair densities, and was affected only slightly by growth of roots with short hairs regardless of their densities (Figure 5).

Decreasing the distance between root tip and the point from which root hairs begin to emerge resulted in increased total depletion volume for root hairs of all lengths and densities under all D_e values (Figure 6). Actual depletion volume was greatest at the highest D_e and shortest tip to first hair distance for all root hair lengths and densities (Figure 7). At the two lower D_e values, as the tip to first root hair distance decreased, roots with long root hairs at high densities increased

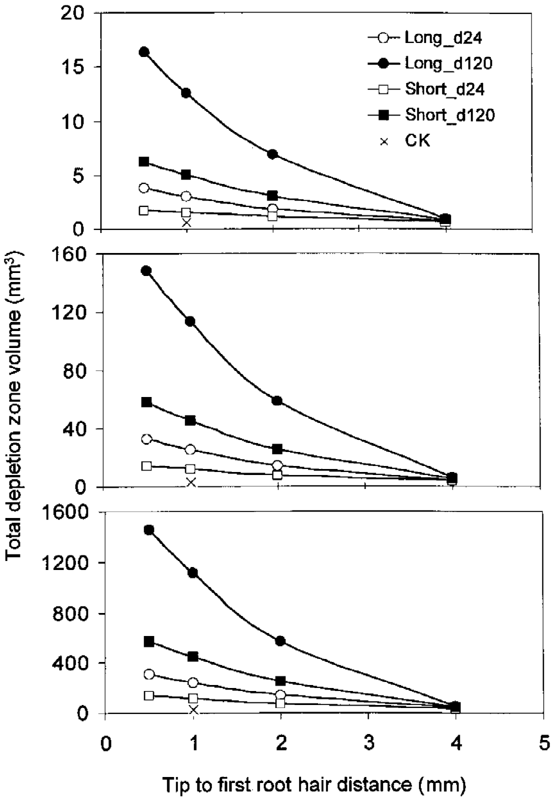

Figure 6. Total depletion volumes of roots with long or short root hairs at either high or low densities as affected by tip to first root hair distances under three diffusion coefficient values (from top down, D_e=1$\times$10^{-9}, 1$\times$10^{-8}, and 1$\times$10^{-7} cm^2 s^{-1}). Control (CK) represents a single root with no root hairs. Data shown as mean $\pm$ standard error of the mean (n = 3). Error bars not shown where their size is smaller than that of the symbols. ANOVA indicates significant effects of the tip to first hair distance (P<0.0001) and its interaction with root hair length (P<0.0001) and density (P<0.0001) under all D_e values. Waller-Duncan K-ratio t-test shows significant differences among different models (K-ratio=100). For D_e=1$\times$10^{-9}, F=72101, critical value of t is 1.80, and minimum significant difference is 0.042. For D_e=1$\times$10^{-8}, F=75456, critical value of t is 1.80, and minimum significant difference is 0.381. For D_e=1$\times$10^{-7}, F=2.856E7, critical value of t is 1.80, and minimum significant difference is 0.194.

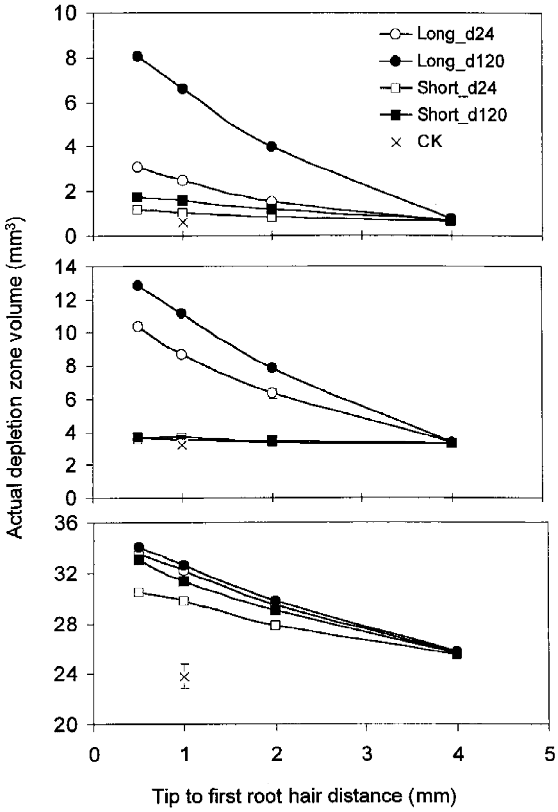

Figure 7. Actual depletion volumes of roots with long or short root hairs at either high or low densities as affected by tip to first root hair distances under three diffusion coefficient values (from top down, D_e=1$\times$10^{-9}, 1$\times$10^{-8}, and 1$\times$10^{-7} cm^2 s^{-1}). Control (CK) represents a single root with no root hairs. Data shown as mean $\pm$ standard error of the mean (n = 3). Error bars not shown where their size is smaller than that of the symbols. ANOVA indicates significant effects of the tip to first hair distance (P<0.0001) and its interaction with root hair length (P<0.0001) and density (P<0.0042) under all D_e values. Waller-Duncan K-ratio t-test shows significant differences among different models (K-ratio=100). For D_e=1$\times$10^{-9}, F=16884, critical value of t is 1.80, and minimum significant difference is 0.0427. For D_e=1 $\times$10^{-8}, F=617, critical value of t is 1.80, and minimum significant difference is 0.339. For D_e=1$\times$10^{-7}, F=149, critical value of t is 1.81, and minimum significant difference is 0.674.

actual depletion volume faster than those with long root hairs at low densities, and those with short root hairs at both low and high densities (Figure 7).

Competition among root hairs

At all D_e values and root hair lengths, competition among root hairs became more intense as root hair densities increased (Figure 8). At any given root hair density, the highest competition among root hairs occurred at D_e= 1$\times$10^{-7} cm^2 s^{-1} and lowest competition at D_e= 1$\times$10^{-9} cm^2 s^{-1}, regardless of root hair length (Figure 8).

At the lowest D_e, competition among root hairs increased over the course of 60 h of growth, with short root hairs at the highest density of 120 having the strongest competition (Figure 9). Length of root hairs did not affect competition among root hairs at low density as much as that at high density as root hairs grew (Figure 9).

Decreased distance between the root tip and the first root hair increased competition among root hairs

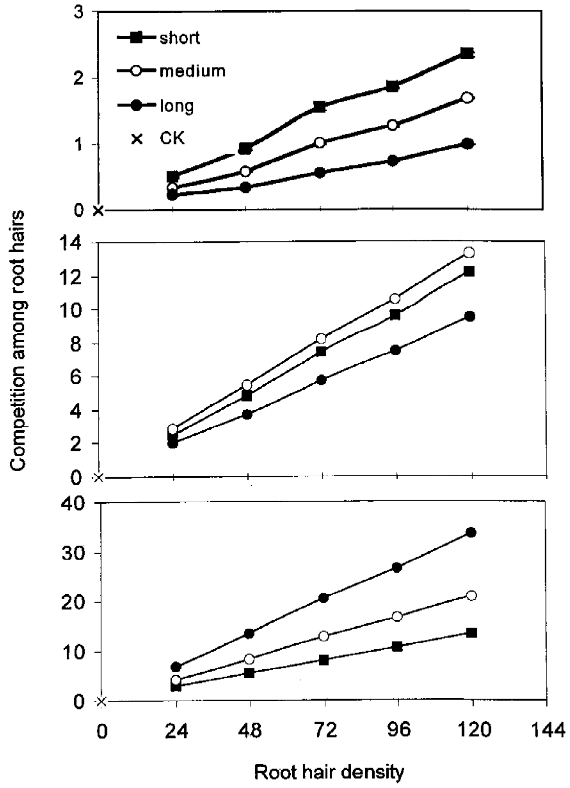

Figure 8. Competition among root hairs at various lengths and densities under three diffusion coefficient values (from top down, D_e=1×10^{-9}, 1×10^{-8}, and 1×10^{-7} cm^2 s^{-1}). Control (CK) represents a single root with no root hairs. Data shown as mean ± standard error of the mean (n = 3). Error bars not shown where their size is smaller than that of the symbols. ANOVA indicates significant effects of root hair length (P<0.0001) and density (P<0.0001), and interaction between the two (P<0.0001) under all D_e values. Waller-Duncan K-ratio t-test shows some significant differences among different models (K-ratio=100). For D_e=1×10^{-9}, F=81, critical value of t is 1.82, and minimum significant difference is 0.214. For D_e=1×10^{-8}, F=2129, critical value of t is 1.81, and minimum significant difference is 0.213. For D_e=1×10^{-7}, F=3924, critical value of t is 1.81, and minimum significant difference is 0.369.

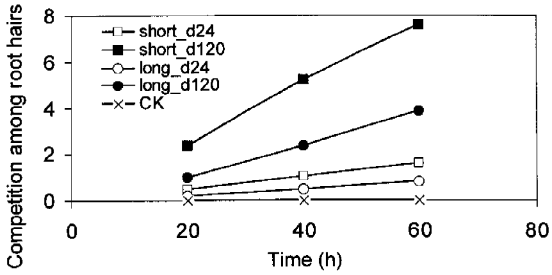

Figure 9. Competition among long and short root hairs at high or low densities under three diffusion coefficient values (from top down, D_e=1×10^{-9}, 1×10^{-8}, and 1×10^{-7} cm^2 s^{-1}) over time. Control (CK) represents a single root with no root hairs. Data shown as mean ± standard error of the mean (n = 3). Error bars not shown where their size is smaller than that of the symbols. ANOVA indicates significant effect of time (P<0.0001) and its interaction with length (P<0.0001) and density (P<0.0001). Waller-Duncan K-ratio t-test shows significant differences among different models at different time points (K-ratio=100): F=12801, critical value of t is 1.81, and minimum significant difference is 0.0498.

at high density much more than that at low density (Figure 10). When D_e= 1×10^{-9} cm^2 s^{-1} and the tip to first root hair distance was shorter than 4 mm, competition among short root hairs was stronger than that among long root hairs at both low and high densities, whereas the opposite was true at D_e= 1×10^{-7} cm^2 s^{-1} (Figure 10).

Phosphorus acquisition efficiency (PAE)

At the highest D_e, in general, phosphorus acquisition efficiency of root hairs decreased as root hair density or length increased (Figure 11). At lower D_e values and for any given root hair density, the PAE of root hairs was in the order of: long hairs > medium length hairs > short hairs (Figure 11). At D_e= 1×10^{-9} cm^2 s^{-1}, PAE was greatest at a density of 96 root hairs for long and medium lengths, and at 96 or 120 for short hairs. At D_e= 1×10^{-8} cm^2 s^{-1}, PAE was greatest at densities of 48 for long hairs, 24 for short hairs, and at either 24 or 48 for root hairs of medium length (Figure 11).

At the lowest D_e, long root hairs were more efficient than short hairs at both the lowest and the highest densities, and increase in root hair density had less effect on the efficiency of short hairs during the first 60 h of growth (Figure 12). Although long root hairs at both the lowest and the highest densities were increasingly more efficient during their growth from 20 h to 40 h, their efficiency began to level off more quickly thereafter at the highest root hair density (Figure 12).

At the lowest D_e, as the tip to first root hair distance decreased, increase in root hair length increased PAE, whereas increase in root hair density increased PAE for long but not for short root hairs (Figure 13). With decreasing tip to first hair distance, the efficiency of short root hairs increased by 80% at a density of 24 and 115% at a density of 120; whereas the efficiency of long root hairs increased by 316% at a density of 24 and 560% at a density of 120 (Figure 13). Similarly, at D_e=1×10^{-8} cm^2 s^{-1}, as the tip to first root hair distance decreased, increase in root hair length increased PAE, whereas changes in density affected long hairs only (Figure 13). One change with increasing

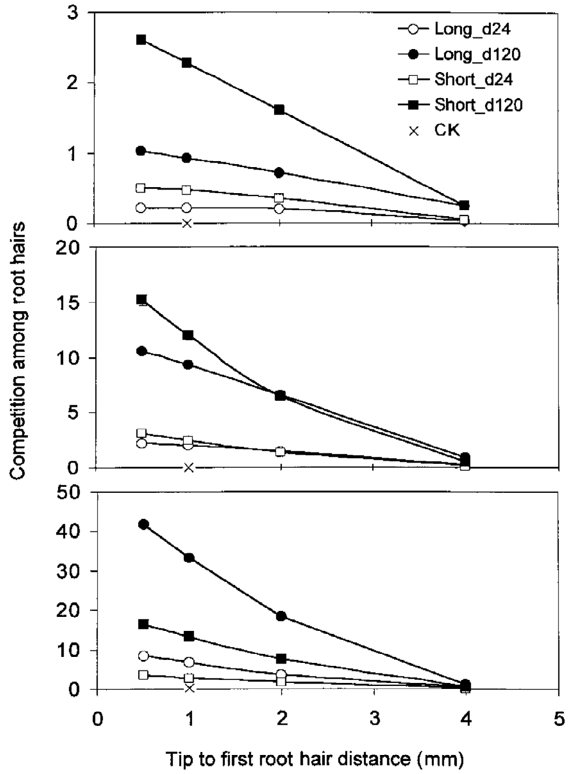

Figure 10. Competition among long or short root hairs at high or low densities as affected by tip to first hair distances and soil diffusion coefficient values (from top down, D_e=1×10^{-9}, 1×10^{-8}, and 1×10^{-7} cm^2 s^{-1}). Control (CK) represents a single root with no root hairs. Data shown as mean ± standard error of the mean (n = 3). Error bars not shown where their size is smaller than that of the symbols. ANOVA indicates significant effects of the tip to first hair distance (P<0.0001) and its interaction with root hair length (P<0.0001) and density (P<0.0001) under all D_e values. Waller-Duncan K-ratio t-test shows significant differences among different models (K-ratio=100). For D_e=1×10^{-9}, F=2509, critical value of t is 1.80, and minimum significant difference is 0.0395. For D_e=1×10^{-8}, F=810.3, critical value of t is 1.80, and minimum significant difference is 0.423. For D_e=1×10^{-7}, F=73128, critical value of t is 1.80, and minimum significant difference is 0.114.

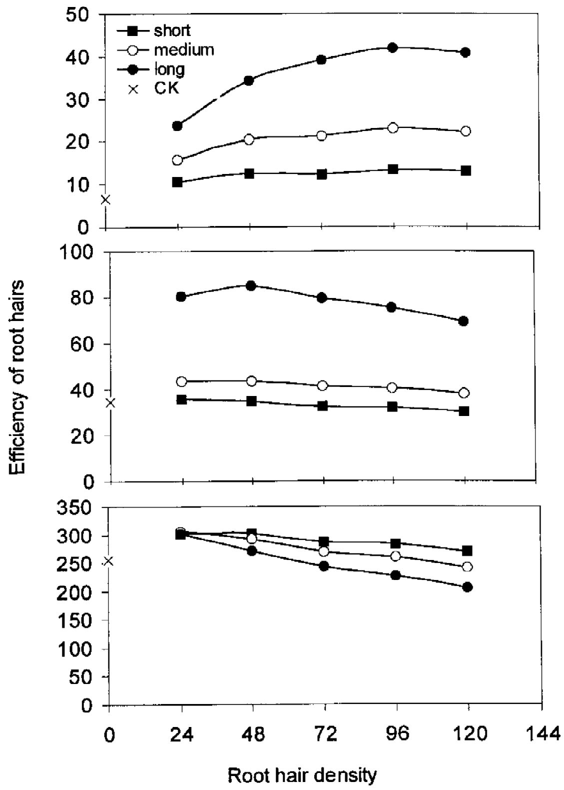

Figure 11. Efficiency of roots with root hairs at various lengths and densities under three diffusion coefficient values (from top down, D_e=1×10^{-9}, 1×10^{-8}, and 1×10^{-7} cm^2 s^{-1}). Control (CK) represents a single root with no root hairs. Data shown as mean ± standard error of the mean (n = 3). Error bars not shown where their size is smaller than that of the symbols. ANOVA indicates significant effects of root hair length (P<0.0001) and density (P<0.0001), and interaction between the two (P<0.0001) under all D_e values. Waller-Duncan K-ratio t-test shows significant differences among different models (K-ratio=100). For D_e=1×10^{-9}, F=4795, critical value of t is 1.81, and minimum significant difference is 0.426. For D_e=1×10^{-8}, F=29328, critical value of t is 1.81, and minimum significant difference is 0.301. For D_e=1×10^{-7}, F=120, critical value of t is 1.81, and minimum significant difference is 6.852.

D_e is that lower density becomes more efficient for both long and short hairs (Figure 13). At the highest D_e, both long and short root hairs were equally most efficient at low density, and the efficiency increased with the decrease in the tip to first root hair distance (Figure 13). In contrast to results at lower values of D_e, at high root hair density, as the tip to first root hair distance decreased, the PAE of long root hairs dropped sharply, whereas for short hairs PAE increased slightly (Figure 13).

Increased root hair density through increasing trichoblast file numbers vs. decreasing trichoblast length

Increasing root hair densities through either increasing the number of trichoblast files or decreasing the trichoblast length both increased the total depletion volume for root hairs of different length (Figure 14). As root hair density increased, actual depletion volume increased as well but more under the lowest D_e (Figure 15).

At D_e= 1×10^{-8} cm^2 s^{-1}, there was little variation in competition resulting from changes in trichoblast

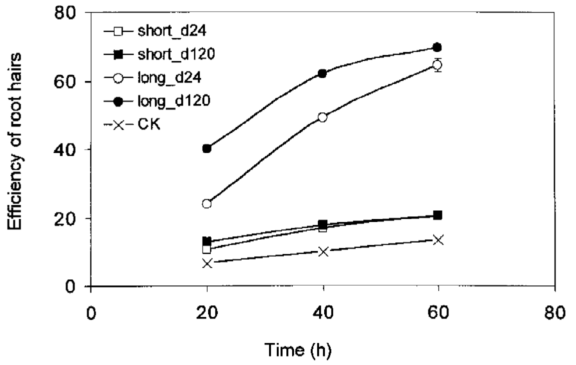

Figure 12. Efficiency through time of roots with long or short root hairs at either high or low densities. D_e=1×10^{-9} cm^2 s^{-1}. Control (CK) represents a single root with no root hairs. Data shown as mean ± standard error of the mean (n = 3). Error bars not shown where their size is smaller than that of the symbols. ANOVA indicates significant effect of time (P<0.0001) and its interaction with length (P<0.0001) and density (P<0.0001). Waller-Duncan K-ratio t-test shows significant differences among different models at different time points (K-ratio=100): F=1633, critical value of t is 1.81, and minimum significant difference is 1.385.

length or file number. As D_e decreased to 1×10^{-9} cm^2 s^{-1}, increased number of trichoblast files resulted in more competition for short hairs and less competition for long hairs relative to decreasing trichoblast length (Figure 16). Patterns in PAE followed those observed with competition. At D_e= 1×10^{-8} cm^2 s^{-1}, there was little difference in PAE resulting from changing trichoblast length or file numbers. At the lowest D_e and longest root hairs, PAE was increased more by increasing the number of trichoblast files than by decreasing trichoblast length (Figure 17). As D_e went up, however, PAE decreased with increased root hair density (Figure 17).

Estimation of synergistic vs. additive effects

Comparison of the observed effects of root hair traits on potential phosphorus acquisition (actual depletion volume) permitted the calculation of trait synergism, i.e. benefits from trait combinations that exceeded merely additive effects (Table 2). Synergistic effects exceeded additive effects by from 101% to over 403% for various combinations of the four traits. For pairs of traits, synergism reached nearly 300% for length and density, and above 300% for length and increased cell files. For combinations of three traits, synergistic effects exceeded additive effects by up to 403%, for the combination of length, density and tip distance. The combination of all four traits resulted in 371% more

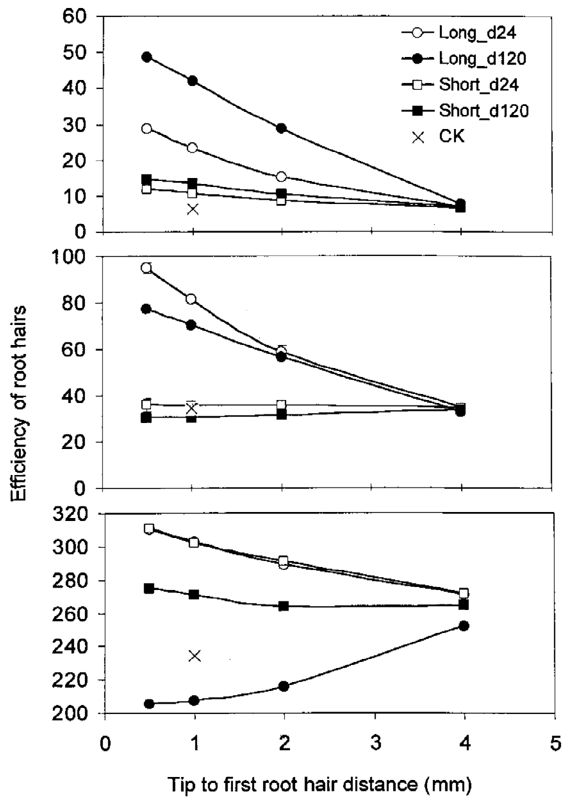

Figure 13. Efficiency of roots with long or short root hairs at high or low densities as affected by tip to first hair distances and soil diffusion coefficient values (from top down, D_e=1×10^{-9}, 1×10^{-8}, and 1×10^{-7} cm^2 s^{-1} Control (CK) represents a single root with no root hairs. Data shown as mean ± standard error of the mean (n = 3). Error bars not shown where their size is smaller than that of the symbols. ANOVA indicates significant effects of the tip to first hair distance (P<0.0001) and its interaction with root hair length (P<0.0001) and density (P<0.0001) under all D_e values. Waller-Duncan K-ratio t-test shows significant differences among different models (K-ratio=100). For D_e=1×10^{-9}, F=7369, critical value of t is 1.80, and minimum significant difference is 0.383. For D_e=1×10^{-8}, F=283.9, critical value of t is 1.80, and minimum significant difference is 3.268. For D_e=1×10^{-7}, F=146.5, critical value of t is 1.81, and minimum significant difference is 7.074.

soil exploration than would be obtained by additive effects alone.

Discussion

The phosphorus acquisition efficiency of single *Arabidopsis* roots, as measured by the ratio of the actual depletion volume for phosphorus to the total root volume, is strongly affected by both soil exploration and the competition among root hairs due to overlap of depletion zones. Single roots without any root hairs

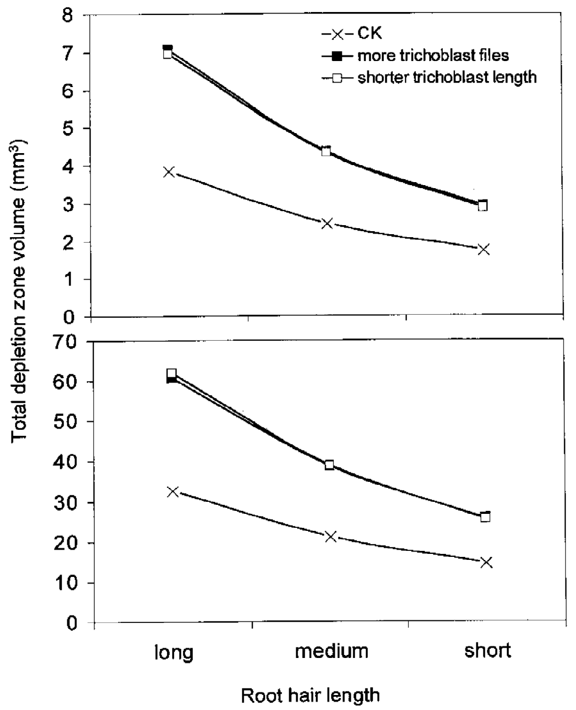

Figure 14. Total depletion volumes of roots at increased root hair densities of 64 either through development of more trichoblast files or decreased trichoblast length, under two diffusion coefficient values (from top down, D_e=1×10^{-9} and 1×10^{-8}). Control (CK) represents a single root with root hairs of density 32. Data shown as mean ± standard error of the mean (n = 3). Error bars not shown where their size is smaller than that of the symbols. ANOVA indicates significant effects of increasing density through either increasing the trichoblast file numbers (P<0.0001) or decreasing trichoblast length (P<0.0001), and their interaction (P<0.0001) under the two D_e values. Waller-Duncan K-ratio t-test shows significant differences among different models (K-ratio=100). For D_e=1×10^{-9}, F=7710, critical value of t is 1.88, and minimum significant difference is 0.057. For D_e=1×10^{-8}, F=7307, critical value of t is 1.88, and minimum significant difference is 0.516.

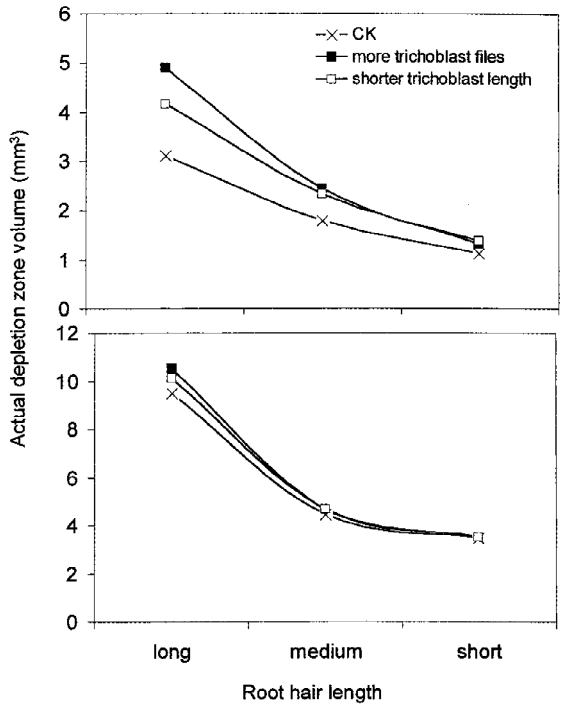

Figure 15. Actual depletion volumes of roots at increased root hair densities of 64 either through development of more trichoblast files or decreased trichoblast length, under two diffusion coefficient values (from top down, D_e=1×10^{-9} and 1×10^{-8}). Control (CK) represents a single root with root hairs of density 32. Data shown as mean ± standard error of the mean (n = 3). Error bars not shown where their size is smaller than that of the symbols. ANOVA indicates significant effects of increasing density through either increasing the trichoblast file numbers (P<0.0001) or decreasing trichoblast length (P<0.0001), and their interaction (P<0.0001) under the two D_e values. Waller-Duncan K-ratio t-test shows significant differences among different models (K-ratio=100). For D_e=1×10^{-9}, F=19193, critical value of t is 1.88, and minimum significant difference is 0.0254. For D_e=1×10^{-8}, F=29290, critical value of t is 1.88, and minimum significant difference is 0.0473.

are less efficient because they explore less soil volume compared to roots with root hairs, that are able to increase the surface area for absorption and extend the spatial exploration of the soil (Figures 2, 3, 8 and 11). When root hairs are dense, intense competition may result in decreased PAE (Figures 8 and 11). However, as root hairs grow longer, overall competition decreases as new soil volume is explored. Therefore, the most efficient soil exploration with the least amount of competition within the root can only be achieved by an optimum synergism between root hair length and density, as shown in Figure 1. Indeed, earlier work on *Arabidopsis* showed high correlation between root hair length and density as a response to phosphorus availability (R^2 = 0.97, Figure 18) (Bates and Lynch, 1996; Ma et al., 2001), and phosphorus acquisition per unit root length is increased with longer root hairs (Bates and Lynch, 2000b). Correlation between root hair length and density was also found in several studies with diverse species (Fohse et al., 1991; Gahoonia et al., 1997, Figure 18).

According to the Nye and Tinker (1977) function used in this study, increasing D_e values will increase the total depletion volume of the root, which also increases with time as the root grows, but at a decreasing rate. In agreement with this prediction and our hypothesis, our simulations showed that total depletion volume was positively correlated with D_e values (Figure 2). However, over the course of 60 h of growth, the rate of increase in total depletion volume did not

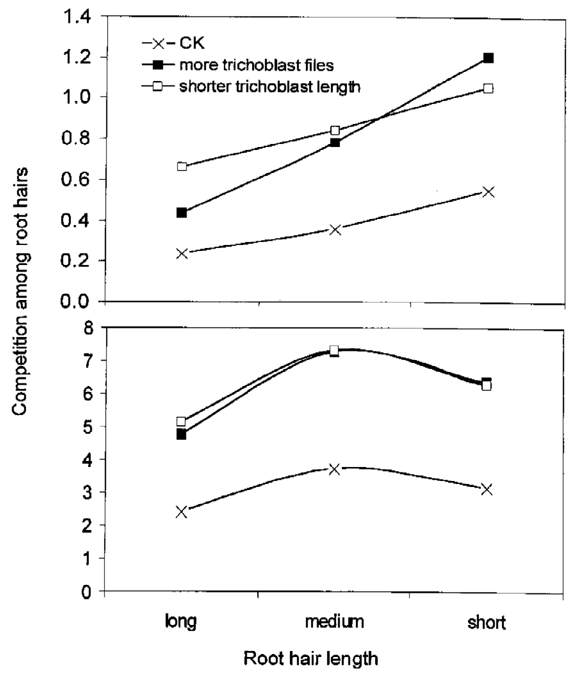

Figure 16. Competition among root hairs at increased root hair densities of 64 either through development of more trichoblast files or decreased trichoblast length, under two diffusion coefficient values (from top down, D_e=1×10^{-9} and 1×10^{-8}). Control (CK) represents a single root with root hairs of density 32. Data shown as mean ± standard error of the mean (n = 3). Error bars not shown where their size is smaller than that of the symbols. ANOVA indicates significant effects of increasing density through either increasing the trichoblast file numbers (P<0.0001) or decreasing trichoblast length (P<0.0001), and their interaction (P<0.0001) under the two D_e values. Waller-Duncan K-ratio t-test shows significant differences among different models (K-ratio=100). For D_e=1×10^{-9}, F=942, critical value of t is 1.88, and minimum significant difference is 0.0279. For D_e=1×10^{-8}, F=5413, critical value of t is 1.88, and minimum significant difference is 0.0648.

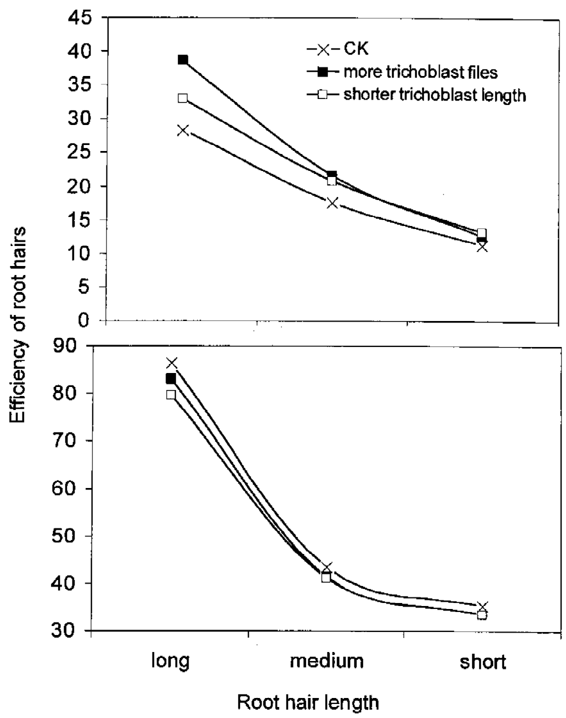

Figure 17. Efficiency of roots with root hairs at increased root hair densities of 64 either through development of more trichoblast files or decreased trichoblast length, under two diffusion coefficient values (from top down, D_e=1×10^{-9} and 1×10^{-8}). Control (CK) represents a single root with root hairs of density 32. Data shown as mean ± standard error of the mean (n = 3). Error bars not shown where their size is smaller than that of the symbols. ANOVA indicates significant effects of increasing density through either increasing the trichoblast file numbers (P<0.0001) or decreasing trichoblast length (P<0.0001), and their interaction (P<0.01) under the two D_e values. Waller-Duncan K-ratio t-test shows significant differences among different models (K-ratio=100). For D_e=1×10^{-9}, F=14837, critical value of t is 1.88, and minimum significant difference is 0.21. For D_e=1×10^{-8}, F=20531, critical value of t is 1.88, and minimum significant difference is 0.424.

decrease (Figure 4). Roots with long root hairs at a density of 120 hairs mm^{-1} continued to expand their total depletion volume about 3 times faster than those with short hairs at the same density, and about 5 – 12 times faster than those with either long or short root hairs at a density of 24 hairs mm^{-1} (Figure 4). This can be attributed to the increase in root volume over time (Figure 19). Had roots stopped increasing in volume, total depletion volume would have started to level off gradually.

We observed that D_e values not only affected total and actual depletion volumes (Figures 2 and 3), but also competition among root hairs (Figure 8), all of which are associated with PAE. The dependence of competition on both root characteristics and the surrounding medium as represented by different D_e values is consistent with the results by Ge et al. (2000). The variations in soil exploration and competition under different D_e values explains the adaptive value of plasticity of root hair length, density and tip to first root hair distance, since the optimal combination of these parameters for PAE will vary with soil conditions (Figure 11). Our data show that the benefit of increased root hair length and density for PAE is more important in soils with lower D_e, as less competition occurs under low D_e values. In contrast, the intense competition among root hairs under high D_e quickly overcomes any benefits from changes in length and density, leading to decreased PAE (Figures 8 and 11).

Table 2. Synergistic vs. additive effects of root hair length, density, tip to first root hair distance, and trichoblast file number on the actual depletion zone volume at D_e=1×10^{-9} cm^2 s^{-1}

Root hair traits	Va (mm^3)	Additive effect (mm^3)	Synergistic effect (mm^3)	Synergistic effect (% over additive)
Control	0.80	——	——	——
L (longer)	1.55	——	——	——
D (denser)	1.25	——	——	——
T (closer to tip)	1.02	——	——	——
F (more files)	1.32	——	——	——
L + D	4.08	1.21	3.28	272
L + T	2.46	0.98	1.67	170
L + F	4.90	1.28	4.11	322
D + T	1.54	0.68	0.74	108
D + F	1.88	0.98	1.08	111
T + F	1.73	0.75	0.93	124
L + D + T	6.57	1.44	5.78	403
L + D + F	4.13	1.73	3.33	192
D + T + F	2.01	1.20	1.21	101
L + D + T + F	8.06	1.96	7.26	371

The additive effects are calculated by subtracting the control value (i.e. actual depletion volume from models with short root hairs (0.29 mm), low density (24), and tip to first root hair distance at 2 mm) from that of each individual trait, then summing up the differences. The synergistic effects are calculated by subtracting the control value from the actual effect of any given combination of root hair traits. L: increase in root hair length from 0.29 to 0.87 mm; D: increase in root hair density from 24 to 120; T: decrease in tip to first root hair distance from 2 to 1 mm; F: increase in number of trichoblast files from 8 to 12.

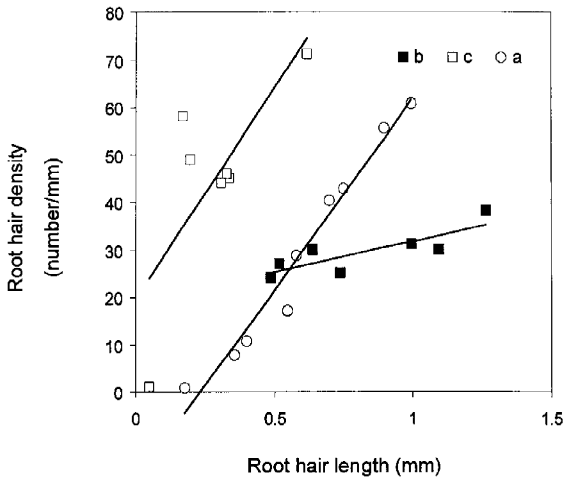

Figure 18. (a) Correlation between root hair length and density in *Arabidopsis* under different phosphorus availabilities. The direction of increased length and density indicates decrease in phosphorus availability. Correlation coefficient R^2 = 0.97. Adapted from Bates and Lynch (1996) and Ma et al. (2001). (b) Correlation between root hair length and density of seven cereal cultivars differing in phosphorus acquisition capacity. Correlation coefficient R^2 = 0.69. Cultivars that had longer root hairs and higher phosphorus acquisition capacity also tended to have more dense root hairs. Adapted from Gahoonia et al. (1997) with permission. (c) Correlation between root hair length and density of onion, ryegrass, wheat, rape, tomato, spinach and bean as related to phosphorus uptake. Correlation coefficient R^2 = 0.55. Adapted from Fohse et al. (1991) with permission.

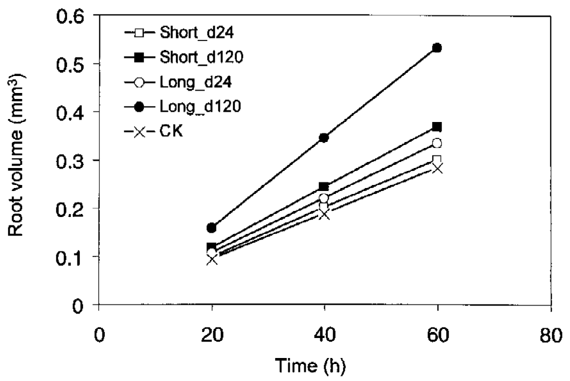

Figure 19. Changes in root volumes over time for long and short root hairs at high or low densities. D_e=1×10^{-9} cm^2 s^{-1}. Control (CK) represents a single root with no root hairs. Data shown as mean ± standard error of the mean (n = 3). Error bars not shown where their size is smaller than that of the symbols.

Our observation that increasing root hair density could lead to decreased phosphorus acquisition efficiency is comparable to that of Fitter et al. (1991) that decreases in root inter-branch distances could cause decreased exploration efficiency when competition is taken into consideration. Given the relationships between the depletion zone radius and root radius, competition and total/actual depletion volume, as well as PAE and actual depletion volume (for references, see Equations (1), (2) and (3) in 'Introduction'), competition among root hairs negatively affects PAE, i.e. high competition is associated with low PAE. As root hair density increases, so does competition (Figure 8). However, since length of root hairs also plays a significant role in soil exploration and competition, the synergism between root hair length and density together determines the PAE of the root. Consider two roots with the same total volume but different root hair configurations, e.g. one with long hairs at low density, and the other with short hairs at high density. Although they have the same total depletion volume (i.e. including overlap in depletion zones), the long haired root will have a greater actual depletion volume than the short haired one, therefore will have less competition and higher PAE, as shown in Figures 2, 3, 8 and 11. This agrees with our hypothesis that the interaction of root hair length and density is important for phosphorus acquisition.

In *Arabidopsis*, increased root hair density under low phosphorus availability was associated with anatomical changes leading to the formation of more trichoblast files (Ma et al., 2001). It is interesting that increased root hair density did not arise from decreased trichoblast length, which could have achieved the same effect on root hair density (Ma et al., 2001). In this study, we simulated models of roots with increased root hair density through either development of more trichoblast files, or shortened trichoblast length. Our data show that at $D_e = 1\times10^{-9}$ cm^2 s^{-1}, while achieving the same increase in root hair density, roots with more trichoblast files had generally larger actual depletion volume, less competition among root hairs, and higher PAE than roots with decreased trichoblast length (Figures 15, 16 and 17).

At the lowest D_e, although total and actual depletion volumes kept increasing over time, and the PAE of long root hairs increased and remained greater than that of short hairs at both low and high densities, the increased competition among root hairs led to the leveling off in PAE of both long and short root hairs at both high and low densities (Figures 4, 5, 9 and 12). This is presumably due to the shape of the depletion volume expanding from a complex shape into a cylindrical shape over time. Root hair geometry may be particularly important for phosphorus acquisition by the apical regions of roots, but may become less important as the root matures.

We have also observed that the distance between the root tip and the first root hairs of wild type *Arabidopsis* plants decreased as phosphorus availability decreased (Zhang et al., unpublished data). This may indicate yet another adaptive response of the root to low phosphorus availability. Our data show that decreased distance between the tip and the most apical root hair increased the total and actual depletion volumes (Figures 6 and 7), and at the same time, increased competition among root hairs (Figure 10). At low D_e values, long root hairs are more efficient as the tip to first root hair distance decreased, whereas at the highest D_e value, the efficiency of long root hairs may actually decrease with shortened tip to first hair distance and increased density (Figure 13). This was due to the intense competition among root hairs as a result of the combined effect of high density and short tip to first hair distance (Figures 8 and 10). This shows that not only is the synergism between root hair length and density important in determining the PAE, but also the distance between root tip and the first root hairs plays a role in the overall synergistic relationship among these parameters.

Given the prevalence of soils with low phosphorus availability, phosphorus acquisition efficiency is likely to be subject to evolutionary selection, but it may not be the only determinant of root plasticity responses. As D_e values decrease the actual depletion volume also decreases. At $D_e = 1\times10^{-9}$ cm^2 s^{-1}, actual depletion volume continually increases with root hair density (Figure 3), while PAE peaks at 96 root hairs per mm. The actual depletion volume may be at least as important in the selection of root hair morphology as PAE. This may be particularly true if the metabolic costs of root hairs are relatively small, as suggested by comparisons of root respiration in wild type and hairless *Arabidopsis* mutants (Bates and Lynch, 2000b).

Our results demonstrate that the responses of *Arabidopsis* root hairs to low phosphorus availability, namely increased root hair length, increased root hair density, shortened tip to first root hair distance, and increased number of trichoblast files (Bates and Lynch, 1996; Ma et al., 2001; Zhang et al., unpublished) are positive adaptations for phosphorus acquisition. Soil exploration as measured by total and actual depletion volumes, and competition among root hairs, are highly dependent on the phosphorus diffusion coefficient. The beneficial effects of these traits for phosphorus acquisition are more pronounced in soils with reduced phosphorus mobility. The combined effects of root hair traits on phosphorus acquisition ranged from 30 to over 400% greater than their additive effects, demonstrating substantial morphological synergy.

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